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Effects of Organic Acid and Bacterial Direct-Feed Microbial on Fattening Performance of Kivircik-Male Yearling Lambs

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Abstract: This study was undertaken to investigate the effects of addition of organic acid (OA), (SAL CURB®) and/or direct-feed microbial (DFM), (Cylactin® LBC ME 10) to the rations of Kivircik male-yearling lambs on body weight gain, feed consumption, feed conversion, some blood metabolites, and the levels of serum minerals. A group of sixty 1-year old male lambs of Kivircik Breed were used. The animals were divided in to 4 groups of 15 lambs each. The groups were assigned as control, organic acid, direct-feed microbial, and direct-feed microbial plus organic acid. A commercial direct-feed microbial product and organic acid mix were used as feed treatment supplements. The average daily gain means of the lambs supplemented with organic acid plus direct-feed microbials was found significantly higher than that of control group lambs. Significant differences in total proteins, cholesterol, total lipids, and serum Zn levels were detected among the groups. As a result, addition of organic acid and/or direct-feed microbial to the ration of yearling Kivircik male lambs resulted in an increased body weight gain ($p<0.05$). In addition, the dietary supplement of organic acid to the lambs caused a decrease in the serum cholesterol level while direct-feed microbial addition caused an increase in the serum Zn level ($p<0.05$).

Key words: Kivircik male lambs, organic acid, direct-feed microbial, performance, blood parameter

Introduction

Recent studies on ruminant nutrition have mostly focused on increasing the feed conversion rate. A variety of feed additives have been developed to achieve this objective. Antimicrobial feed additives, which are one of the additives that have been used widely in the world; have been prohibited or restricted in most countries due to the increased concern of causing resistance to antibiotics in bacterial pathogens. Therefore, the interest to potential alternatives to antibiotic feed additives such as direct-feed microbials (DFM), which is known as biological products composed of cultures of useful microorganisms (Alp and Kahraman, 1996; Krehbiel *et al.,* 2003; Elam *et al.,* 2003), and organic acids increased (Callaway and Martin, 1996; Martin, 1998; Castillo *et al.,* 2004).

In ruminants, direct-feed microbials are mainly used for lowering the effects of stress conditions and decreasing the use of antibiotics in calves. These microbials both increase milk yield in dairy cows and the body weight gain and feed conversion rate in beef cattle (Krehbiel *et al.,* 2003). Lema *et al.* (2001) it has been reported that the addition of direct-feed microbials to the ration of sheep resulted in decreased numbers of harmful microorganisms in the intestines improved fattening performance, and feed conversion rate.

Few studies have been carried out on the action of DFM on blood parameters and published results have been conflicted. Jouany *et al.* (1998), reported that DFM did not have any effect on ruminal metabolism such as protein decomposition or microbial protein synthesis and did not cause any changes in some of the blood parameters including serum urea, total protein and albumin. In contrast, Chiofalo *et al.* (2004), found an increase in serum urea concentration, whereas no change was observed in blood total protein and total cholesterol.

There is still no product or method developed as a substitute of antimicrobial feed additives. Yet, organic acids (OA) are thought to be as one of the most important candidate as an alternative of antibiotic feed additives.

Organic acids establish antimicrobial effect in the intestines by suppressing fungal activity and maintaining acidic environment (Dibner and Buttin, 2002). Organic acids are reported to improve rumen fermentation, like ionophor antibiotics, they do and maintain the rumen pH even after consumption of carbohydrate-rich feeds through which an increased growth and fattening performance are observed (Martin, 1998). Martin *et al.,*

Table 1: Concentrate composition of the experimental ration.

Ingredient	g/kg, as mixed
Corn grain, ground	360.0
Barley grain, ground	200.0
Sunflower Meal (280 g/kg CP)	200.0
Corn bran	80.0
Wheat bran	70.0
Molasses	65.0
Limestone	18.0
Salt	5.0
Vitamin + mineral premix*	2.0

*Composition of vitamin - mineral premix per kilogram of premix: vitamin A 12 000 000 IU; vitamin D_3 3 000 000 IU; vitamin E 30 g; Mn 50 g; Fe 50 g; Zn 50 g; Cu 10 g; I 0.85 g; Co 0.15 g; Se 0.15 g.

Table 2: Nutrient contents of diet (DM basis)

Chemical composition (g/kg)	Concentrate*	Forage**
Dry Matter	872.4	834.6
Ash	61.0	96.4
Crude protein (N X 6.25)	148.4	104.2
Crude fiber	96.1	337.0
Ether extract	22.3	15.6
Ca	8.1	6.9
P	4.7	1.5

*Ration consisted of 1: 1 concentrate: forage. **Composition of per kilogram of forage: grass hay 520 g, wheat straw 440 g and corn silage 40 g, as fed.

1999, noted that plasma component concentrations were generally unaffected by organic acid (DL-malate) except that, plasma urea level was lower for steers whereas cholesterol was higher for heifers. Results of a recent study by Castillo *et al.* (2004) showed that, in addition to buffering effect in feed and rumen, organic acids might increase energy-efficiency and digestibility of crude protein, calcium, and phosphorus by lowering methane production and decrease the numbers of harmful bacteria attached to the intestinal wall.

The organic acid and DFM are feed supplements to ruminant with the claim of improving performance. However, there is little information about OA and DFM or their combination on performance of lambs. To further investigate the efficacy of using this additives with along or their combination for lambs, we conducted experiment to compare the effects of OA and DFM or their combination on performance and blood parameters of Kivircik male-yearling lambs. Blood metabolite concentrations were also evaluated for nutritional status of lambs.

Materials and Methods

Sixty yearling Kivircik male lambs were used in the present study. The animals were randomly divided into four boxes of four different treatments. The four different treatments were assigned as control (C, no additive), organic acid (OA), direct-feed microbial (DFM), and organic acid and direct-feed microbial combination (OA+DFM). The direct-feed microbial used was a commercial product of *Enterococcus faecium cernelle 68* strain (Cylactin® LBC ME 10) at $1.0x10^{10}$ cfu/g concentration. The organic acid used was a commercial mixture (SAL CURB® brand dry) of 1 g/kg (propionic acid, calcium propionate, ammonium formate, sorbic acid, formic acid, and BHA (butylated hydroxyanisole). The concentrate of control group of animals did not receive any additive. The animals in OA group received a mixture of organic acids at 3 g/kg, group DFM animals received direct-feed microbial at 1 g/kg, and OA+DFM group of animals received a combination of organic acid (1.5 g/kg) and direct-feed microbial (0.5 g/kg) in their concentrates.

The treatment ration consisted of 1:1 forage: concentrate and was fed as total mixed ration (Table 1 and 2). Fresh and clean drinking water and feed (mixture of roughage and concentrate-yearling lamb feed) were given to the animals *ad libitum*.

The roughage and concentrate feed were analyzed for dry matter, ether extract, Nitrogen, and crude ash using standard procedures AOAC (1990). Dry matter was determined by oven drying for 24 h at 105°C (DM; 934.01), Nitrogen using the Kjeldahl Procedure with a Kjeltec- UDK 126 A (Velp Scientific, Italy) (CP; 954.01), crude ash by combustion at 550°C for 6h (OM; 942.01) and ether extract by using Soxhlet extraction procedure. Each animal was ear-tagged and weighed on days 0, 28, and 56 using a scale. Feed intake was recorded daily as the difference between what was offered and what was refused. In addition, the feed conversion ratio of every group was calculated. Health conditions of the lambs were closely monitored during the study.

Blood samples were collected from the individual animals after morning feedings on days 0, 28, and 56. The blood samples were taken from V. jugularis in to 10 ml-vacutainer tubes without anticoagulant and centrifuged (4000rpm/min) for the separation of serum. Using ready-to-use spectrophotometrical kits, the serum samples were analyzed for deterring the levels of albumin (Chema Diagnostica BC 0500 CH), total protein (Chema Diagnostica TP 0500 CH), urea (Chema Diagnostica A2 F245 CH), cholesterol (Chema Diagnostica CT F400), total lipids (Spinreact, 1001270), Ca (Spinreact, 1001060), Mg (Chema Diagnostica TP 0500 CH), and Pi (Spinreact, 1001155). In addition, serum Fe, Cu and Zn levels were determined using atomic absorption spectrophotometer (AAS, Shimadzu 680 AA) according to the methods of AOAC (1990).

The data was analyzed for One-way variance analysis (ANOVA) using SPSS statistical analysis software (SPSS, 2002). Tukey's HSD test was used for comparing the means of the treatment groups. A significant level of 0.05 was used for comparisons (Snedecor and Cochran, 1980). Statistical analysis were not conducted for feed consumption and feed conversion ratios since the animals were fed in-groups.

Table 3: Body weight and average daily weight gain of yearling lambs

	Groups				
	C	OA	DFM	OA+DFM	SEM
Body weight, kg					
Initial	47.90	46.03	48.03	44.75	1.010
Day 28	51.97	51.52	52.21	51.31	1.090
Day 56	59.90	59.75	61.49	59.52	1.134
Average daily weight gain, g					
Day 0 - 28	145.1^{b}	195.9^{ab}	149.1^{b}	234.4^{a}	10.40**
Day 29 - 56	283.2^{b}	294.2^{ab}	331.5^{a}	293.2^{b}	9.11*
Day 0 - 56	214.3^{b}	244.9^{ab}	240.4^{ab}	263.9^{a}	7.33*

Groups: Control (C); Organic Acid (OA); Direct-fed microbials (DFM); Organic acid + Direct-fed microbials (OA+DFM)

$^{a-b)}$ Means within sub rows with no common superscripts differ significantly (p<0.05). * p<0.05; **p<0.01

Results

The effect of dietary organic acid supplement and direct-feed microbial on body weight (BW) and average daily gain (ADG) of Kivircik Breed yearling male lambs are shown in Table 3. Significant differences in ADG between periods of days 0-28, 29-56, and 0-56 were detected among the groups. However, during the trial, the animals of OA, P, or P+OA groups showed no significant change regarding body weight at day 56. Overall ADG of the lambs in OA+P group were found significantly higher than that of control group the trough d 56.

Feed consumption for different periods (days 0-28, 29-56 and 0-56) for total, roughage, and concentrate and calculated amount of feed for 1 kg of BW gain is provided in Table 4. Statistical analysis were not conducted for feed consumption and feed conversion ratios since the animals were fed in groups.

The data of the effect on lipid, protein and mineral metabolism created by adding organic acid and/or direct-feed microbial to the rations of Kivircik yearling lambs are presented in Table 5. Significant differences were found among groups at the levels of urea, cholesterol, Mg, and Zn on day 28 and at the levels of total proteins, cholesterol, total lipids and Zn on day 56.

Discussion

In this study, it has been investigated how the addition of organic acid and/or direct-feed microbial to the rations of Kivircik Breed male yearling lambs affect the feeding performance, some blood parameters. The feed additives are used in animal nutrition either by direct addition to feed or water or as silage additive (Alp and Kahraman 1996; Kurtoglu *et al.,* 2001). In recent years, a variety of *in vitro* rumen studies have been undertaken to investigate the effects of OA and DFM additives on rumen metabolism (Newbold *et al.,* 1995; Callaway and Martin, 1996; Lopez *et al.,* 1999; Agarwal *et al.,* 2000).

Results of the present study indicated that there were appreciable but not significant differences among groups in mean BW of the animals determined at the end of the feeding period. However, the ADG of the animals, which received OA, DFM, or OA+DFM were significantly higher regarding in their rations than daily gains of the control animals during the entire study period (Table 3). For instance, BW gain of the animals in OA+DFM group per day was 23.15% higher than that of control group lambs (p<0.05). Although the daily feed consumption of the animals in all groups were close to each other, feed conversion ratios determined in animals that received feed additive in their rations were higher than those determined in control lambs (Table 4). The results of the present study are consistent with other published reports indicating that addition of direct-feed microbial as microbial feed additive to the ration of sheep resulted in increased BW gain and feed conversion rate (Lubbadeh *et al.,* 1999; Lema *et al.,* 2001; Chiofalo *et al.,* 2004). Henderson *et al.* (1986) reported that feeding a sheep with silage inoculated through bacterial cultures resulted in an increased consumption of dry substances and increased ADG compared to the control group animals. In a similar study done by Emanuelle *et al.* (1992) feeding the lambs with inoculum-added dry forage improved the feed consumption, BW gain, and feed conversion rate of the animals.

Results of a study carried out by Nadeau *et al.* (2000) revealed that bacterial inoculum and formic acid added silage resulted in higher dry matter intake and improved growth performance. In another study, it was indicated that the addition of organic acid to the feed of steers improved the ADG by 8.6% and enhanced the growth performance (Martin *et al.,* 1999).

The data, which were on the effects of direct-feed microbials added to the feed, might be due to differences in cultures used as direct-feed microbial as well as variations in breed, age, gender, and environmental conditions, as explained by Krehbiel *et al.* (2003).

Results of the analysis of the serum samples obtained from blood samples taken on day 28 and 56 of the study are presented Table 5. Blood metabolite concentrations were evaluated for nutritional status of Kivircik male yearling lambs.

Regarding to the parameters concerning lipid metabolism, the result of the present study was that the

Table 4: Feed consumption and feed efficiency ratio of yearling lambs

	Concentrate				Forage				Total			
	Groups*											
	C	OA	DFM	OA+ DFM	C	OA	DFM	OA+ DFM	C	OA	DFM	OA+ DFM
Feed Consumption (gram per day)												
Day 0 - 28	1046	1056	1049	1052	975	1001	975	985	2020	2057	2023	2036
Day 29- 56	1107	1106	1107	1124	1049	1045	1049	1059	2156	2151	2155	2183
Day 0 - 56	1073	1078	1075	1083	1008	1021	1007	1018	2080	2099	2081	2101
Feed Efficiency (gain: feed)												
Day 0 - 28	0.14	0.19	0.14	0.22	0.15	0.20	0.15	0.24	0.07	0.09	0.07	0.11
Day 29- 56	0.26	0.27	0.30	0.26	0.27	0.28	0.32	0.28	0.13	0.14	0.15	0.13
Day 0 - 56	0.20	0.23	0.22	0.24	0.21	0.24	0.24	0.26	0.10	0.12	0.12	0.13

* Groups: Control (C); Organic Acid (OA); Direct-fed microbials (DFM); Organic acid + Direct-fed microbials (OA+DFM)

Table 5: Effects of organic acid and/or direct-fed microbials on some blood parameters in yearling lambs

	Groups				SEM
	C	OA	DFM	OA + DFM	
Initial					
Albumin (g/L)	37.90	38.40	34.70	35.00	0.69
Total protein (g/L)	65.40	62.70	61.30	64.50	1.25
Urea (g/L)	0.26	0.20	0.27	0.28	0.02
Cholesterol (g/L)	0.86	0.91	0.82	0.78	0.02
Total lipid (g/L)	7.46	7.17	8.09	7.39	0.13
Ca (g/L)	0.09	0.09	0.10	0.10	0.003
Mg (mEq/L)	58.30	43.10	66.10	47.90	3.21
P_i (g/L)	0.05	0.04	0.05	0.05	0.002
Fe (mg/L)	1.20	1.20	1.30	1.30	0.53
Cu (mg/L)	0.70	0.71	0.61	0.78	0.03
Zn (mg/L)	0.61	0.54	0.64	0.53	0.02
Day 28					
Albumin (g/L)	40.50	38.10	41.90	42.30	0.82
Total protein (g/L)	62.10	55	65.40	62.40	2.15
Urea (g/L)	0.18^{b}	0.28^{a}	0.16^{b}	0.21^{ab}	0.01*
Cholesterol (g/L)	7.69^{a}	5.95^{b}	8.24^{a}	7.42^{ab}	0.03**
Total lipid (g/L)	7.11	7.39	7.33	6.91	0.11
Ca (g/L) 0.09	0.08	0.08	0.07	0.003	
Mg (mEq/L)	53.4^{b}	70.5^{a}	68.0^{ab}	52.1^{b}	2.50**
P_i (g/L)	0.04	0.05	0.05	0.04	0.001
Fe (mg/L)	1.4	1.3	1.4	1.2	0.05
Cu (mg/L)	0.98	0.86	0.96	0.93	0.03
Zn (mg/L)	0.85^{ab}	0.69^{c}	0.91^{a}	0.77^{bc}	0.02***
Day 56					
Albumin (g/L)	40.20	38.90	39.70	39.50	0.90
Total protein (g/L)	88.30^{a}	61.60^{b}	63.60^{b}	65.90^{b}	2.89***
Urea (g/L)	0.20	0.24	0.19	0.22	0.01
Cholesterol (g/L)	9.60^{a}	7.23^{b}	8.41^{ab}	7.98^{b}	0.22**
Total lipid (g/L)	8.02^{a}	7.20^{ab}	7.33^{ab}	6.70^{b}	0.15*
Ca (g/L) 0.10	0.08	0.08	0.09	0.003	
Mg (mEq/L)	45.10	52.10	52.20	55	3.10
P_i (g/L)	0.04	0.03	0.04	0.03	0.002
Fe (mg/L)	1.80	1.70	1.80	1.80	0.06
Cu (mg/L)	0.93	0.94	0.88	0.84	0.02
Zn (mg/L)	0.64^{b}	0.84^{a}	0.86^{a}	0.85^{a}	0.03

** Groups: Control (C); Organic Acid (OA); Direct-fed microbials (DFM); Organic acid + Direct-fed microbials (OA+DFM)

$^{a-c)}$ Means within sub rows with no common superscripts differ significantly ($p<0.05$). *$p<0.05$; ** $p<0.01$; *** $p<0.001$

addition of the OA to the rations of the lambs significantly decreased the cholesterol level of the OA group animals on days 28 and 56. On the other hand, the addition of organic acid and direct-feed microbial decreased the total lipids relatively but not significantly. The addition of OA+DFM decreased the level of lipids significantly on day 56.

Although there are number of studies indicating that direct-feed microbials lower the blood cholesterol level (Alp and Kahraman 1996; Lubbadeh *et al.*, 1999), the results of the current study revealed that organic acids decreased significantly the cholesterol level more than direct-feed microbials did. Lubbadeh *et al.,* (1999) brought up a possible explanation why the direct-feed microbials decrease the level of cholesterol. Microbial feed additives reduce the absorption of lipid from the intestines by deconjugation; consequently it may decrease blood cholesterol.

The total protein, urea and albumin contents in blood are indicators of protein metabolism in the organism. In this study, the urea level of the yearling lambs in OA group was found higher on day 28 than other groups ($p<0.05$). This higher concentration of plasma urea was due to increased quantity of ammonia absorbed from rumen and circulating in the plasma because of the increased feed consumption. Total protein level of the control group animals was also found significantly higher than the other groups at the end of the study period.

In one of the study, the effect of direct-feed microbials on rumen metabolism and fattening performance in sheep was observed and it was found that DFM did not change the levels of some blood parameters including urea, total proteins, and albumin. As a result, N metabolism was not affected by yeast supplementation (Jouany *et al.,* 1998). In contrast to our result, Martin *et al.* (1999) noted that blood urea concentration was decreased by organic acid supplementation. The reduction in plasma urea level for steers might have resulted from more efficient utilization of dietary nitrogen. In addition, Chiofalo *et al.* (2004) concluded that the application of direct-feed microbial diet to growing Maltase goat kids decreased blood total protein and urea levels. The authors claimed that the lower content of urea could be justified by better nutritional status.

During the trial, mineral metabolism was affected by treatment. On day 28 of the present study; the serum Mg level of the lambs in OA group was found significantly higher than those of the control and OA+DFM groups. At the end of this study (day 56), however, there was no significant difference in Mg levels between the groups. Another finding of the present study is that the addition of direct-feed microbial to the ration caused and increased serum Zn level ($p<0.05$).

The factors affecting mineral needs of animals, inorganic composition of the body and tissues are determined by species of the animal, race, age, gender, growth rate, health condition, nutritional condition, endocrinological condition, season, and physiological condition (lactation, pregnancy, dry period). In addition, the mineral composition of the body and tissues depend on levels of mineral and protein of the diet (i.e., amount, chemical form, interaction between minerals) (Alp *et al.,* 2001; Kahraman *et al.,* 2002). Abu-Damir *et al.,* 1991, reported that diet-induced change in blood acid-base status was an important factor for mineral retention in lambs and the rates of mineral retention were lowered by acid diets in lambs.

Conclusions: Results of the present study indicated that addition of organic acid or direct-feed microbial alone or their combination to the rations of Kivircik Breed -male yearling lambs increased body weight gain. Furthermore, organic acid addition to the ration caused a decrease in blood cholesterol level while the addition of direct-feed microbial caused an increase in blood Zn level.

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